

Serological Evidence of Glanders and Brucellosis in Equines in The North-East of Algeria

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Abstract

Burkholderia mallei and *Brucella spp.* are two bacteria of non-Gram-stained bacilli, non-sporulated, unencapsulated and which are responsible for glanders and brucellosis respectively. In addition, equines could play an important epidemiological role in the dissemination of these diseases. In 2016, a study was undertaken in clinically healthy donkeys (n = 120) and horses (n = 119) in 3 departments in the North-East of Algeria, in order to determine the seroprevalence of glanders and brucellosis. The Complement Fixation Test was used to detect specific antibodies against *Burkholderia mallei* and in association with Rose Bengal Plate Test (RBPT) for specific antibodies against *Brucella spp.* No positive cases were observed for glanders; concerning brucellosis, an individual seroprevalence was estimated at 0% in donkeys and 0.8 (± 2.5)% in horses with only one positive case found in the department of El Taref. This first serological study in Algerian equines has shown the absence of glanders and a very low seropositive distribution of brucellosis through study area. However, further investigations are needed with much more samples in other regions to better understand these diseases and establish an adequate prophylaxis program adapted to the Algerian epidemiological situation.

Keywords: *Burkholderia mallei*, *Brucella spp.*, Glanders, brucellosis, donkey, horse, RBPT, CFT, Algeria

INTRODUCTION

Some bacterial diseases in domestic equines such as donkeys, mules and horses are transmissible to humans by direct contact with infected animals and/or with contaminated materials. In this regard, arise glanders and brucellosis as two zoonosis which may endanger human health [1, 2].

Previously called *Pseudomonas mallei*, *Burkholderia mallei* is the bacterium that causes glanders [3]. It could take an acute form in donkeys and mules with death in few days. In horses, it takes generally a chronic form and animals can survive for years. Chronic and subclinical 'occult' cases are potential sources of infection due to the permanent or intermittent shedding of bacteria [4]. In chronic form, symptoms may be late in appearance and may be observed only after several weeks or even months. The symptomatology can be quite rough and the disease goes disregarded. Animals with chronic or subclinical occult forms are dangerous sources of infection, and could represent the most infectious cases [5].

Brucella is a Gram-negative bacterium which causes the zoonotic disease brucellosis, it occurs worldwide and has been found in a wide range of domestic and wild mammals [6]. Ruminants represent the primary reservoir of this organism, but different species of equines are less susceptible to the infection by *B. abortus* and *B. suis* causing a bursitis and swelling of the neck or back; abortions also are possible [7]. Aborted material and vaginal discharges of infected cattle and swine could be responsible of the bacterial spread to equines [5]. Generally, the disease is asymptomatic in ruminants, but it can cause reproduction disorders. These reproductive failures are accompanied with shedding of high levels of bacteria into vaginal excretion, birth products, milk and semen of infected animals [7]. Concerning human infection, it results mainly after a physical contact [8].

According to the literature, glanders has been eradicated from many countries, but it is still reported in the African,

Asian, Australian and Latin American continents [9]. At the opposite, brucellosis is endemic in most areas of the world, except some industrialized countries where the disease is completely eradicated [10]. In Algeria, the first description of glanders goes back to 1841 by Guyon and other cases since 2000 [11], at the opposite, brucellosis in equine has never been reported in our knowledge.

In order to determine the serological prevalence of glanders and brucellosis in equines in natural exposition, a study was conducted in 2015. This study will provide an important assessment in order to compare future serological surveys.

MATERIALS AND METHODS

STUDY SITE

The study took place in 3 departments (Skikda, Annaba and El Taref) in north-east of Algeria (Figure 1). These regions are well placed to perform a study for exploring the epidemiology of glanders and equine brucellosis because of the high numbers of donkeys and horses. In addition, these regions represent an area of commercial transit for a neighboring country which is Tunisia.

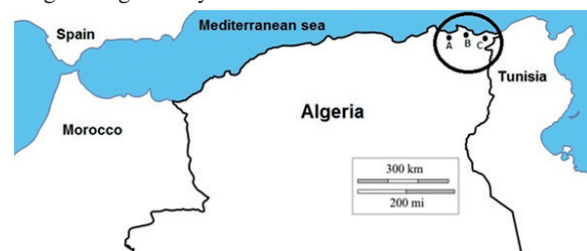


Figure 1. Geographical representation of study area
 A: Department of Skikda B: Department of Annaba
 C: Department of El Taref

SAMPLES COLLECTION (SAMPLING)

Donkeys and horses of both sexes are included in the study belonging to different owners. A survey was compiled to provide information regarding animals (sex, age). A blood sample (5 mL) was collected by veinpuncture of each animal into a vacutainer tube. Each tube was marked with a code including an individual sampling number and accompanied by an information sheet. The sera were separated from precipitate blood by centrifugation at 1,500×g for 15 min, aliquoted into clean 1.5 mL plastic tubes and stored at -20°C until tested. All samples that show hemolysis were discarded from the study.

LABORATORY TESTING (SURVEY METHOD)

The analyses were done at the LBRA (Laboratory of Animal Reproduction Biotechnologies) at the Veterinary Institute, University Blida 1.

Glanders

Complement Fixation Test (CFT).

The antigen used consists primarily of lipopolysaccharides of cell wall. All recommended controls to check test conditions were included. The sample that showed no hemolysis is considered as positive.

Brucellosis

Rose Bengal Plate Test (RBPT). The presence of *Brucella spp.* antibodies in the serum samples was detected using a commercially available Rose Bengal Plate Test (RBPT) antigen according to the method described by Alton et al. (1988) [12]. The technique uses a stained antigen with Rose Bengal and buffered to a low pH, in our case, produced by Brucellosis National Reference Center, Spain.

Complement Fixation Test (CFT) was carried out in a 96 well microtiter plates, we used 25 µL of twofold dilutions of

inactivated sera, antigen from S99 *B. abortus* strain, guinea pig complement, and different concentrations of fresh or preserved sensitized sheep red blood cells. Reference negative and positive sera were used in all serological confirmation tests. Serum samples with antibody titers of 30 or greater ICFTU/mL are usually considered to be positive.

STATISTICAL ANALYSIS

Data were analyzed using EPI-INFO® 6.04 software [Centers for Disease Control and Prevention, USA]. A Fisher exact test was used to detect significant differences between sampling areas, species, sex and ages of animals. A probability of less than 5 % was considered statistically significant. Statistical uncertainty was assessed by calculating the 95% confidence interval for each proportion.

RESULTS AND DISCUSSION

The principal aim of this study was to determinate the seroprevalence of glanders and brucellosis in donkeys and horses. As shown in Table 1, no positive or doubtful cases were observed for glanders in both tested species. Concerning brucellosis, no positive case was observed for donkeys among 120 tested (0%), for horses, two positive cases were observed with RBPT. After confirmation with CFT, only one serum gave a titer equivalent to 40 ICFTU/mL which is considered as positive. The positive case has been observed in the department of El Taref with a rate of 1.2 (±3.6)% between 82 tested, among 119 horses tested with a rate of 0.8 (±2.5)%, among 124 females tested with a rate of 0.8 (±2.4)%. The age of this female was 8 years, with a rate of 1.2 (±3.6)%. This female has no antecedent abortion history or other reproductive disorder.

Table 1. Main results of seroprevalence with different variables

Variables		Glanders		Brucellosis	
		Positive cases	Negative cases	Positive cases	Negative cases
Regions	Skikda	0	72	0	72
	Annaba	0	75	0	75
	El Taref	0	82	1	81
Species	Donkey	0	120	0	120
	Horse	0	119	1	118
Sex	Male	0	115	0	115
	female	0	124	1	123
Ages	[1- 5[0	65	0	65
	[5-10[0	83	1	82
	[10-15[0	78	0	78
	15 and more	0	16	0	13
Total	/	0	229	/	228

The seroprevalence did not differed significantly ($p>0.05$) according to species, regions, sex and ages of tested animals.

The CFT has been used for glanders' diagnosis especially in populations with very low glanders prevalence, despite its specificity which has been questioned [13]. Several industrialized countries have been able to eradicate glanders, such as the United States Canada and the West of Europe [14]. The absence of positive cases in both species tested in our study cannot confirm the status of this disease since it was last reported in 2000 [11]. However, the resurgence of glanders in some countries has been reported for horses in EAU [15] ; India [16] and Pakistan [17] and for donkeys in Brazil [18]. This in fact, always obliges us to keep the vigilance degree in a high level since the subclinical carriers could be introduce the disease into free regions at any seasonal period [4].

Because the culture of *Brucella spp.* is very dangerous and necessitates a laboratory with high level of biosecurity, brucellosis is usually diagnosed by serological tests [19]. The Rose Bengal Plate Test (RBPT) has a good sensitivity especially in the detection of IgG1 comparing to the Slide Agglutination Test (SAT), in spite of the false positive reactions which can appear after cross reactions with antibodies of some bacteria [20, 21]. The CFT is the method officially accredited for serological brucellosis diagnosis because of its excellent specificity [22].

The knowledge about the distribution of equine brucellosis is based on serological surveys and close seroprevalence rates to our study have already been reported somewhere else in donkeys : 2,1% in Sudan [23], 5% in Nigeria [10] and horses: 0% in Eriteria [19], 0.2% in Mexico [24], 0.05% [25] and 2.5% [7] in Iran. On the other side, high seroprevalence rates have been observed in different countries like Egypt with 54,2% in donkeys [26], Pakistan with 20.7% [27], Nigeria with 14,7% [21] and Mongolia with 11,6% for horses [28]. However, animals involved in our survey were without any apparent symptoms, which can partly justify the low number of positive cases. Another probable reason is the method of rearing horses and donkeys, which is practiced away from dairy cows, which repel a possible direct contamination.

Our results can only be interpreted as an evidence of a previous infection or an ancient exposure. In some cases, the RBPT test gives a false-positive result reaction because of the antigenic community with other bacteria [10].

There was no evidence of any relationship between regions, species, sex and ages of tested animals, probably according to the weakness of sampling, while other studies have shown the predominance of the infection in females [21].

According to the geographical data, our results of seroprevalence for equine brucellosis indicate that the degree of invasion in the study area is very low and has no a zoonotic potential. Because the infection is prevalent in equines cohabiting with cattle and a herd becomes infected with *Brucella* when infected animals are introduced into it [5], we think that the incidence of equine brucellosis could be higher in other departments where bovine herds are in high numbers and bovine brucellosis is enzootic [29] and an implication of animal brucellosis in human cases is confirmed [30]. At all, the choice of clinically sound animals could partly explain the absence of positive cases for glanders and the observation of a single positive case of brucellosis.

Our results indicate that no evidence of glanders has been reported in our study and the degree of equine brucellosis invasion is very low. Because the number of laboratories

where glanders and brucellosis can be diagnosed are limited; a surveillance plan with further analysis should be conducted in the future for detecting *Burkholderia mallei* and *Brucella spp.* early as possible. It is desirable to conduct surveys with a greater number of horses, donkeys and mules and include other species in the same regions. The declaration of the human cases of one of these diseases must be accompanied by a very thorough survey on the context of contact with any equine species. Molecular biology is of significant utility in confirming the involvement of these animals in the zoonotic aspect of these diseases. We hope that this will help to establish a good prophylactic plan adapted to Algerian context.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

REFERENCES

- [1] Corbel MJ, Banai M. 2005. Genus I. *Brucella* Meyer and Shaw 1920. In: Brenner DJ, Krig NR, Staley JT edition, *Bergey's manual of systematic bacteriology*. Springer, New York.
- [2] Van Zandt KE, Greer MT, Gelhaus HC. 2013. Glanders: an overview of infection in humans. *Orphanet Journal of Rare Diseases*, 8:131.
- [3] Dvorak GD, Spickler AR. 2008. Glanders. *Journal of the American Veterinary Medical Association*, 233[4]:570-577.
- [4] Wittig MB, Wohlsein P, Hagen RM, Al Dahouk S, Tomaso H, Scholz HC, Nikolaou K, Wernery R, Wernery U, Kinne J, Elschner M, Neubauer H. 2006. Glanders-a comprehensive review. *Deutsche tierärztliche Wochenschrift* [9]:323-330.
- [5] Khan I, Wieler LH, Melzer F, Elschner MC, Muhammad G, Ali S, Sprague LD, Neubauer H, Saqib M. 2013. Glanders in animals: A review on epidemiology, clinical presentation, diagnosis and countermeasures. *Transmitted Emerging Diseases*, 60: 204-221.
- [6] Gul ST, Khan A. 2007. Epidemiology and epizootology of Brucellosis: a review. *Pakistan Veterinary Journal*, 27: 145-151.
- [7] Tahamtan Y, Namavari MM, Mohammadi G, Jula GM. 2010. Prevalence of brucellosis in horse north-east of Iran. *Journal of Equine Veterinary Science*, 30: 376-387.
- [8] Erganis O, Hadimli HH, Solmaz H, Corlu M. 2005. Comparison of rose Bengal plate test antigens prepared from *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. *Bulletin of the Veterinary Institute of Pulawy*, 49: 165-167.
- [9] 2015. OIE: Chapter 2.1.12. q fever, in: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, Birds and Bees)*, 8th ed., O.I.E editions http://web.oie.int/eng/normes/MANUAL/2008/pdf/2.05.11_GLANDERS.pdf.
- [10] Sadiq MA, Tijjani A, Auwal MS, AR Mustapha, I Gulani. 2013. Serological prevalence of brucellosis among donkeys (*Equus asinus*) in some local government areas of Yobe State, Nigeria. *Journal of Equine Veterinary Science*, 33: 150-154.

- [11] CDC. 2015. Etymologia: Glanders. *Emerging Infectious Disease*, 21[1]:47.
- [12] Alton GG, Jones LM, Angus RD, Verger GM. 2015. *Techniques for the brucellosis Laboratory*. Paris: Institut National de la Recherche Agronomique, 190, 1988.
- [13] Sprague LD, Zachariah R, Neubauer H, Wernery R, Joseph M, Scholz HC, Wernery U. 2009. Prevalence-dependent use of serological tests for diagnosing glanders in horses. *BMC Veterinary Research*, 5: 32.
- [14] Derbyshire JB. 2002. The eradication of glanders in Canada. *Canadian Veterinary Journal*, 43[9]: 722-726.
- [15] Scholz HC, Joseph M, Tomaso H, Al Dahouk S, Witte A, Kinne J, Hagen RM, Wernery R, Wernery U, Neubauer H. 2006. Detection of the reemerging agent *Burkholderia mallei* in a recent outbreak of glanders in the United Arab Emirates by a newly developed fliP-based polymerase chain reaction assay. *Diagnosis of Microbiology and Infectious Disease*, 54[4]:241-247.
- [16] Malik P, Singha H, Khurana SK, Kumar R, Kumar S, Raut AA, Riyesh T, Vaid RK, Virmani N, Singh BK, Pathak SV, Parkale DD, Singh B, Pandey SB, Sharma TR, Chauhan BC, Awasthi V, Jain S, Singh RK. 2012. Emergence and re-emergence of glanders in India: a description of outbreaks from 2006 to 2011. *Veterinaria Italiana*, 48[2]: 164-167.
- [17] Hornstra H1, Pearson T, Georgia S, Liguori A, Dale J, Price E, O'Neill M, Deshazer D, Muhammad G, Saqib M, Naureen A, Keim P. 2017. Molecular Epidemiology of Glanders, *Pakistan. Emerging Infectious Diseases*, 15[12]: 2036-2039.
- [18] Mota RA, da Fonseca Oliveira AA, da Silva AMV, de Farias B, Rabelo SSA. 2010. Glanders in Donkeys (*Equus Asinus*) in the State of Pernambuco, Brazil: A Case Report. *Brazilian Journal of Microbiology*, 41 [1]: 146-149.
- [19] Getachew T, Getachew G, Sintayehu G, Getenet M, Fasil A. 2016. Bayesian Estimation of Sensitivity and Specificity of Rose Bengal, Complement Fixation, and Indirect ELISA Tests for the Diagnosis of Bovine Brucellosis in Ethiopia. *Veterinary Medicine International*, PMC 4993953.
- [20] Omer MK, Skjerve E, Holstad G, Woldehiwet Z, Macmillan AP G. 2000. Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. *Epidemiology and Infection*, 125: 447-453.
- [21] Ehizibolo DO, Gusi AM, Ehizibolo PO, Mbuk EU, Ocholi RA. 2011. Serologic prevalence of brucellosis in horse stables in two northern states of Nigeria. *Journal of Equine Science*, 22[1]: 17-19.
- [22] OIE. 2009. Bovine Brucellosis and Caprine and Ovine Brucellosis: Chapter 2.4.3.-2.7.2 *Manual of Diagnostic Tests and Vaccines*. World Organization for Animal Health 35.
- [23] Abdalla MA, Abdalla SH, Elzaki R. 2010. Prevalence of *Brucella abortus* antibodies in donkeys in Gedaref state of eastern Sudan. In: *Tropentag*; September 14-16, Zurich, Switzerland.
- [24] Acosta-Gonzalez RI, Gonzalez R, Flores-Gutierrez GH. 2000. Prevalence of *Brucella abortus* antibodies in equines of a tropical region of Mexico. *Canadian Journal of Veterinary Research*, 70: 302-304.
- [25] Ghobadi N, Salehi AR. 2013. Evaluation prevalence of brucellosis in horse Hamadan of Iran. *Inter J Agr Crop Sci*, 6: 603-605.
- [26] Esmat M, El-Mezyen AF. 1983. Studies on fistulous withers in donkeys. *Egyptian Journal of Applied Science*, 8: 453-465.
- [27] Wadood F, Ahmad M, Khan A, Gul TS, Rehman N. 2009. Seroprevalence of brucellosis in horses in and around Faisalabad. *Pakistan Veterinary Journal* 2009; 29: 196-198.
- [28] Zolzaya B, Selenge T, Narangarav T, Gantsetseg D, Erdenechimeg D, Zinsstag J, Schelling E. 2014. Representative seroprevalences of human and livestock brucellosis in two Mongolian provinces, *Ecohealth*, 11:356-371.
- [29] Kabouia R, Khaled H, Kerrou M, Bouazziz O, Belmerzoug M. 2014. Epidemiological study of bovine brucellosis in area Constantine, *Annals of Biological Research*, 5[2]: 38-41.
- [30] Lounes N, Cherfa M A, Le Carrou G, Bouyoucef A, Jay M, Garin-Bastuji B, Mick V. 2014. Human Brucellosis in Maghreb: Existence of a Lineage Related to Socio-Historical Connections with Europe, *PLOS ONE*, 0115319.